

Km^R. Infectious phage particles can be obtained by use of helper phage. As explained below, the gene III signal sequence is capable of directing (BPTI):-
:(mature-gene-III-protein) to the surface of phage. In
5 M13-MB51, we have inserted DNA encoding gene VIII coat protein (50 amino acids) and three stop codons 5' to the DNA encoding the mature gene III protein.

Summary of signal peptide fusion protein variants.

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	Promoter	RBS	Signal sequence	Fusion protein
	<u>tac</u>	new	<u>VIII</u>	BPTI/VIII-coat
	<u>tac</u>	new	<u>phoA</u>	BPTI/VIII-coat
15	<u>amp</u>	<u>amp</u>	<u>amp</u>	BPTI/VIII-coat
	<u>III</u>	<u>III</u>	<u>III</u>	BPTI/VIII-coat

ANALYSIS OF THE PROTEIN PRODUCTS
ENCODED BY THE SYNTHETIC

20 (signal-peptide::mature-BPTI::VIII-coat-protein) GENES

i) In vitro analysis

25 A coupled transcription/translation prokaryotic system (Amersham Corp., Arlington Heights, IL) was utilized for the in vitro analysis of the protein products encoded by the BPTI/VIII synthetic gene and the variants derived from this.

30 Table 107 lists the protein products encoded by the listed vectors which are visualized by the standard method of fluorography following in vitro synthesis in the presence of ³⁵S-methionine and separation of the products using SDS polyacrylamide gel electrophoresis.
35 In each sample a pre-beta-lactamase product (approx-

imately 31 kd) can be seen. This is derived from the amp gene which is the common selection gene for each of the vectors. In addition, a (pre-BPTI/VIII) product encoded by the synthetic gene and variants can be seen as indicated. The migration of these species (approximately 16kd) is consistent with the expected size of the encoded proteins.

ii) In vivo analysis.

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The vectors detailed in sections (B) and (C) were freshly transfected into the E. coli strain XL1-blue (TM) (Strategene, La Jolla, CA) and in strain SEF'. E. coli strain SE6004 (LISS85) carries the prlA4 mutation and is more permissive in secretion than strains that carry the wild-type prlA allele. SE6004 is F⁻ and is deleted for lacI; thus the cells can not be infected by M13 and lacUV5 and tac promoters can not be regulated with IPTG. Strain SEF' is derived from strain SE6004 (LISS85) by crossing with XL1-Blue(TM); the F' in XL1-Blue(TM) carries Tc^R and lacI^q. SE6004 is streptomycin^R, Tc^S while XL1-Blue(TM) is streptomycin^S, Tc^R so that both parental strains can be killed with the combination of Tc and streptomycin. SEF' retains the secretion-permissive phenotype of the parental strain, SE6004(prgA4).

The fresh transfectants were grown in NZYCM medium (MANI89) for 1 hour after which IPTG was added over the range of concentrations 1.0 micromolar to 0.5 millimolar (to derepress the lacUV5 and tac promoters) and grown for an additional 1.5 hours.

Aliquots of the bacterial cells expressing the synthetic insert encoded proteins together with the

appropriate controls (no vector, vector with no insert and zero IPTG) were lysed in SDS gel loading buffer and electrophoresed in 20% polyacrylamide gels containing SDS and urea. Duplicate gels were either silver
5 stained (Daiichi, Tokyo, Japan) or electrotransferred to a nylon matrix (Immobilon from Millipore, Bedford, MA) for western analysis by standard means using rabbit anti-BPTI polyclonal antibodies.

10 Table 108 lists the interesting proteins visualized on a silver stained gel and by western analysis of an identical gel. We can see clearly in the western analysis that protein species containing BPTI epitopes are present in the test strains which are absent from
15 the control strains and which are also IPTG inducible. In XL1-Blue(TM), the migration of this species is predominantly that of the unprocessed form of the pro-protein although a small proportion of the encoded proteins appear to migrate at a size consistent with
20 that of a fully processed form. In SEF', the processed form predominates, there being only a faint band corresponding to the unprocessed species.

Thus in strain SEF', we have produced a tripartite
25 fusion protein that is specifically cleaved after the secretion signal sequence. We believe that the mature protein comprises BPTI followed by the gene VIII coat protein and that the coat protein moiety spans the membrane. We believe that it is highly likely that one
30 or more copies, perhaps hundreds of copies, of this protein will co-assemble into M13 derived phage or M13-like phagemids. This construction will allow us to a) mutagenize the BPTI domain, b) display each of the variants on the coat of one or more phage (one type per
35 phage), and c) recover those phage that display

variants having novel binding properties with respect to target materials of our choice.

5 Rasched and Oberer (RASC86) report that phage
produced in cells that express two alleles of gene
VIII, that have differences within the first 11
residues of the mature coat protein, contain some of
each protein. Thus, because we have achieved in vivo
processing of the phoA(signal)::bpti::matureVIII fusion
10 gene, it is highly likely that co-expression of this
gene with wild-type VIII will lead to production of
phage bearing BPTI domains on their surface. Mutagen-
esis of the bpti domain of these genes will provide a
population of phage, each phage carrying a gene that
15 codes for the variant of BPTI displayed on the phage
surface.

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20 Example 3

CONSTRUCTION OF BPTI/GENE-III DISPLAY VECTOR

25 DNA manipulations were conducted according to
standard procedures as described in Maniatis et al.
(MANI82). The construction of the geneIII expression
vector is outlined in Figure 12. First the unwanted
lacZ was removed. M13-MB1/2 RF was cut with BamHI and
SalI and the large fragment was isolated by agarose gel
30 electrophoresis. The recovered 6819 bp fragment was
filled in with Klenow fragment of E. coli DNA poly-
merase and ligated to a synthetic HindIII 8mer linker
(CAAGCTTG). The ligation sample was used to transfect
competent XL1-Blue(TM) cells which were subsequently
35 plated for plaque formation. RF DNA was prepared from